

Supplementary Material

TITLE

Structural Insights of a PI3K/mTOR Dual Inhibitor with the morpholino-triazine scaffold

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Table S1-1 SAR-table (core structure)

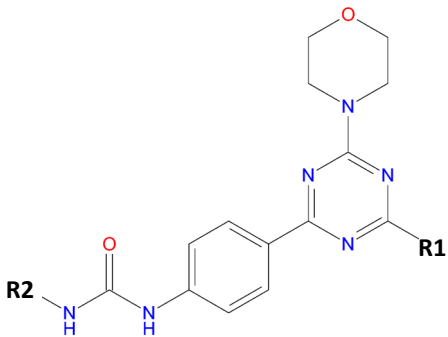
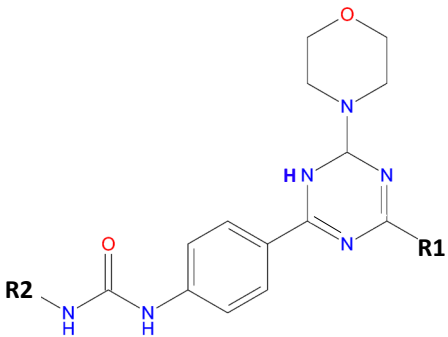
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Table S1-2 SAR-table (continued)

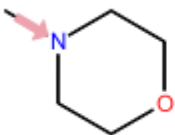
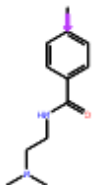
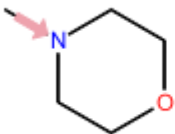
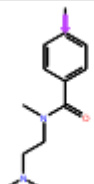
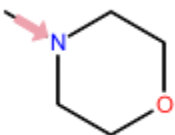
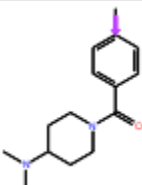
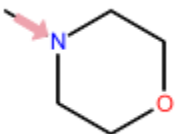
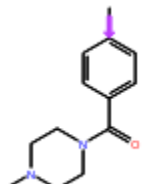
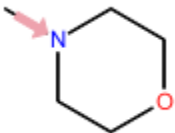
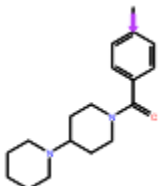
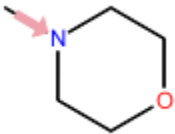
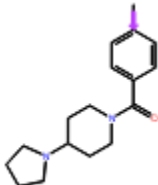
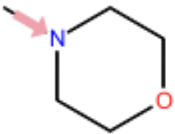
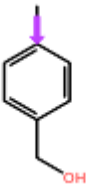
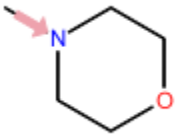
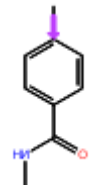
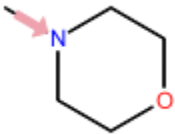
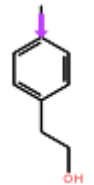
| Structure Name | R1 | R2 | Core Number | pIC50 PI3K | pIC50 mTOR |
|----------------|---|---|-------------|------------|------------|
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| 45379226 |  |  | 1 | 9.40 | 9.22 |
| 44516953 |  |  | 1 | 9.40 | 8.80 |
| 45379224 |  |  | 1 | 9.30 | 8.85 |
| 44516636 |  |  | 1 | 9.22 | 9.15 |
| 44516635 |  |  | 1 | 9.15 | 9.10 |
| 44515084 |  |  | 1 | 9.00 | 8.72 |
| 44516310 |  |  | 1 | 8.82 | 9.15 |
| 44515083 |  |  | 1 | 8.82 | 8.74 |

Table S1-3 SAR-table (continued)

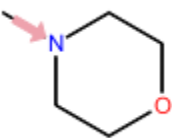
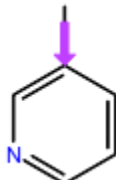
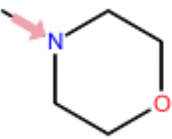
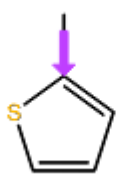
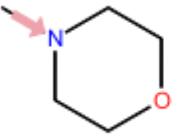
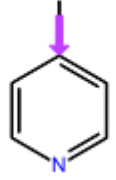
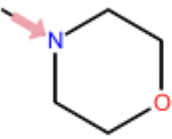
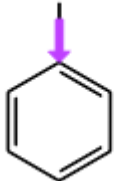
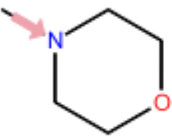
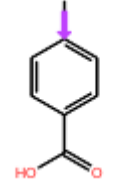
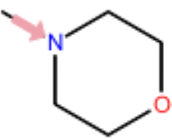
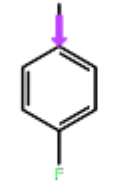
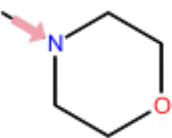
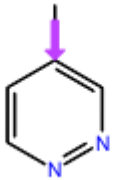
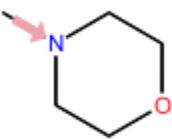
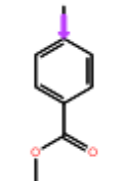
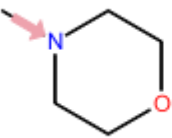
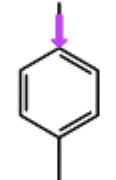
| Structure Name | R1 | R2 | Core Number | pIC50 PI3K | pIC50 mTOR |
|----------------|---|---|-------------|------------|------------|
| 44513885 |  |  | 1 | 8.77 | 8.92 |
| 44513887 |  |  | 1 | 8.70 | 8.77 |
| 44513884 |  |  | 1 | 8.52 | 8.92 |
| 44513886 |  |  | 1 | 8.52 | 8.41 |
| 44516307 |  |  | 1 | 8.22 | 8.82 |
| 44514201 |  |  | 1 | 8.19 | 8.41 |
| 45379223 |  |  | 1 | 7.96 | 8.64 |
| 44515696 |  |  | 1 | 7.90 | 8.60 |
| 44513888 |  |  | 1 | 7.78 | 8.20 |

Table S1-4 SAR-table (continued)

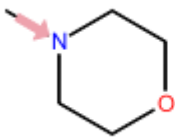
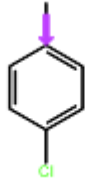
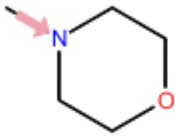
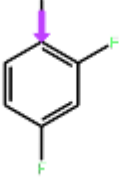
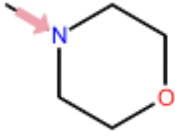

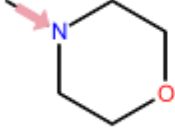

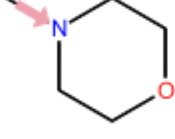
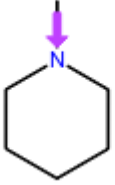
| Structure Name | R1 | R2 | Core Number | pIC50 PI3K | pIC50 mTOR |
|----------------|---|---|-------------|------------|------------|
| 44514203 |  |  | 1 | 7.71 | 8.17 |
| 44514205 |  |  | 1 | 7.22 | 8.03 |
| 44514481 |  |  | 1 | 6.61 | 8.00 |
| 45379222 |  |  | 1 | 6.46 | 7.88 |
| 46228569 |  |  | 1 | 5.82 | 6.34 |

Table S1-5 SAR-table (continued)

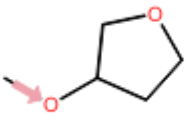
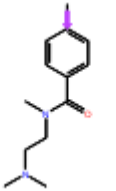
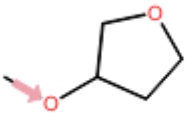
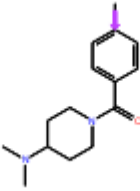
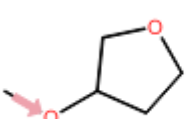
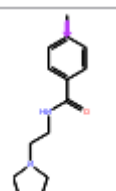
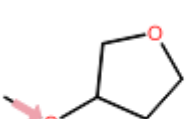
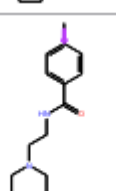
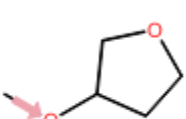
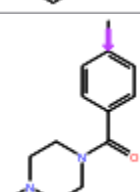
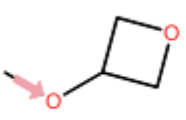
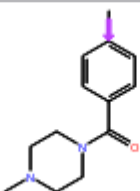
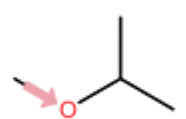
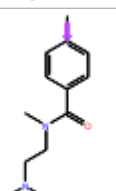
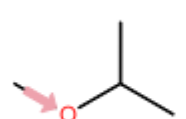
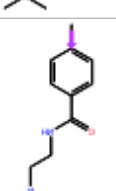
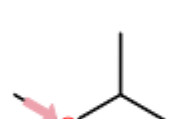
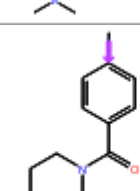
| Structure Name | R1 | R2 | Core Number | pIC50 PI3K | pIC50 mTOR |
|----------------|---|---|-------------|------------|------------|
| 53379513 |  |  | 1 | 9.70 | 9.15 |
| 56683917 |  |  | 1 | 9.00 | 8.96 |
| 56660109 |  |  | 1 | 8.85 | 9.52 |
| 56673952 |  |  | 1 | 8.85 | 9.40 |
| 56683918 |  |  | 1 | 8.77 | 8.77 |
| 56660108 |  |  | 1 | 9.22 | 8.52 |
| 44514208 |  |  | 1 | 9.00 | 9.52 |
| 44514210 |  |  | 1 | 8.52 | 9.40 |
| 44517288 |  |  | 1 | 8.52 | 8.89 |

Table S1-6 SAR-table (continued)


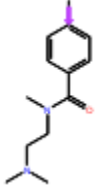

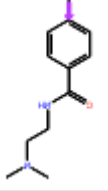

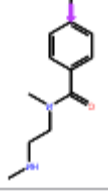

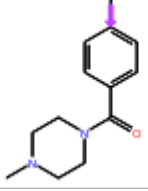

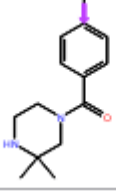

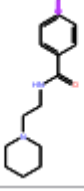
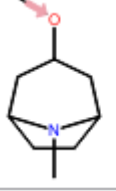
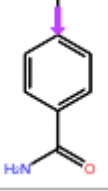

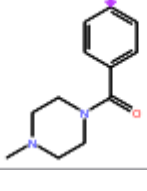
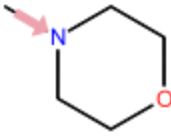
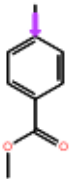
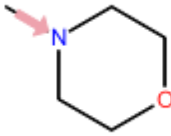
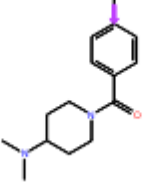

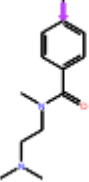
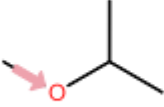
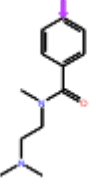
| Structure Name | R1 | R2 | Core Number | pIC50 PI3K | pIC50 mTOR |
|----------------|---|---|-------------|------------|------------|
| 56667023 |  |  | 1 | 9.40 | 8.11 |
| 56677282 |  |  | 1 | 9.22 | 8.25 |
| 56683916 |  |  | 1 | 9.10 | 7.84 |
| 56677283 |  |  | 1 | 8.92 | 7.65 |
| 56663573 |  |  | 1 | 8.85 | 7.73 |
| 56670464 |  |  | 1 | 8.70 | 8.09 |
| 44548482 |  |  | 1 | 8.49 | 8.28 |
| 56683919 |  |  | 2 | 7.82 | 5.92 |

Table S2 Examples of activity cliffs

(A)

| Structure Name | R1 | R2 | Core Number | pIC50 PI3K | pIC50 mTOR |
|----------------|---|---|-------------|------------|------------|
| 44515696 |  |  | 1 | 7.90 | 8.60 |
| 44516953 |  |  | 1 | 9.40 | 8.80 |

(B)

| Structure Name | R1 | R2 | Core Number | pIC50 PI3K | pIC50 mTOR |
|----------------|---|---|-------------|------------|------------|
| 56667023 |  |  | 1 | 9.40 | 8.11 |
| 44514208 |  |  | 1 | 9.00 | 9.52 |

(C)

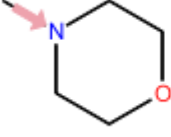
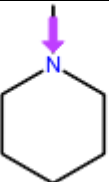
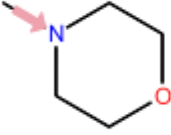
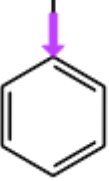
| Structure Name | R1 | R2 | Core Number | pIC50 PI3K | pIC50 mTOR |
|----------------|---|---|-------------|------------|------------|
| 46228569 |  |  | 1 | 5.82 | 6.34 |
| 44513886 |  |  | 1 | 8.52 | 8.41 |

Table S3 Observed and predicted activities

| CID | PI3K | | mTOR | |
|----------|-------------------|-----------|-------------------|-----------|
| | observed | predicted | observed | predicted |
| 44473371 | 9.40 ^a | 9.66 | 9.30 ^a | 9.30 |
| 44513884 | 8.52 | 8.14 | 8.92 | 8.74 |
| 44513885 | 8.77 | 8.28 | 8.92 | 8.17 |
| 44513886 | 8.52 | 8.18 | 8.41 | 8.22 |
| 44513887 | 8.70 | 8.26 | 8.77 | 8.55 |
| 44513888 | 7.78 | 8.29 | 8.20 | 8.72 |
| 44514201 | 8.19 | 8.25 | 8.41 | 8.65 |
| 44514203 | 7.71 | 8.30 | 8.17 | 8.71 |
| 44514205 | 7.22 | 7.31 | 8.03 | 8.13 |
| 44514208 | 9.00 | 9.05 | 9.52 ^a | 9.36 |
| 44514210 | 8.52 | 9.10 | 9.40 ^a | 9.42 |
| 44514481 | 6.61 | 7.69 | 8.00 | 8.04 |
| 44515083 | 8.82 | 7.78 | 8.75 | 8.24 |
| 44515084 | 9.00 | 7.69 | 8.72 | 8.80 |
| 44515696 | 7.90 | 7.50 | 8.60 | 8.36 |
| 44516307 | 8.22 | 8.36 | 8.82 | 8.51 |
| 44516310 | 8.82 | 8.57 | 9.16 | 8.36 |
| 44516635 | 9.16 | 9.20 | 9.10 | 9.14 |
| 44516636 | 9.22 | 9.35 | 9.16 | 8.96 |
| 44516953 | 9.40 ^a | 9.27 | 8.80 | 8.68 |
| 44517288 | 8.52 | 8.50 | 8.89 | 9.01 |
| 44548482 | 8.50 | 8.40 | 8.28 | 8.95 |
| 45379222 | 6.46 ^b | 7.02 | 7.88 | 7.91 |
| 45379223 | 7.96 | 8.16 | 8.64 | 8.70 |
| 45379224 | 9.30 ^a | 8.66 | 8.85 | 8.93 |
| 45379226 | 9.40 ^a | 9.41 | 9.22 | 8.67 |
| 46228569 | 5.82 ^b | 6.35 | 6.34 ^b | 6.41 |
| 53379513 | 9.70 ^a | 8.95 | 9.16 | 9.33 |
| 56660108 | 9.22 | 8.95 | 8.52 | 8.43 |
| 56660109 | 8.85 | 9.47 | 9.52 ^a | 9.54 |
| 56663573 | 8.85 | 8.78 | 7.73 | 7.73 |
| 56667023 | 9.40 ^a | 9.60 | 8.11 | 7.99 |
| 56670464 | 8.70 | 8.96 | 8.09 | 8.17 |
| 56673952 | 8.85 | 9.01 | 9.40 ^a | 8.55 |
| 56677282 | 9.22 | 9.39 | 8.25 | 8.34 |
| 56677283 | 8.92 | 8.50 | 7.65 | 8.54 |
| 56683916 | 9.10 | 9.02 | 7.84 | 8.17 |
| 56683917 | 9.00 | 8.93 | 8.96 | 8.06 |
| 56683918 | 8.77 | — | 8.77 | — |
| 56683919 | 7.82 | — | 5.92 ^a | — |

a: most active compound

b: least active compound

Table S4 QSAR coefficient

| | | average | sd |
|---------------|----------------|---------|------|
| PI3K α | Q ² | 0.65 | 0.08 |
| | R ² | 0.74 | 0.05 |
| | | average | sd |
| mTOR | Q ² | 0.56 | 0.09 |
| | R ² | 0.69 | 0.04 |

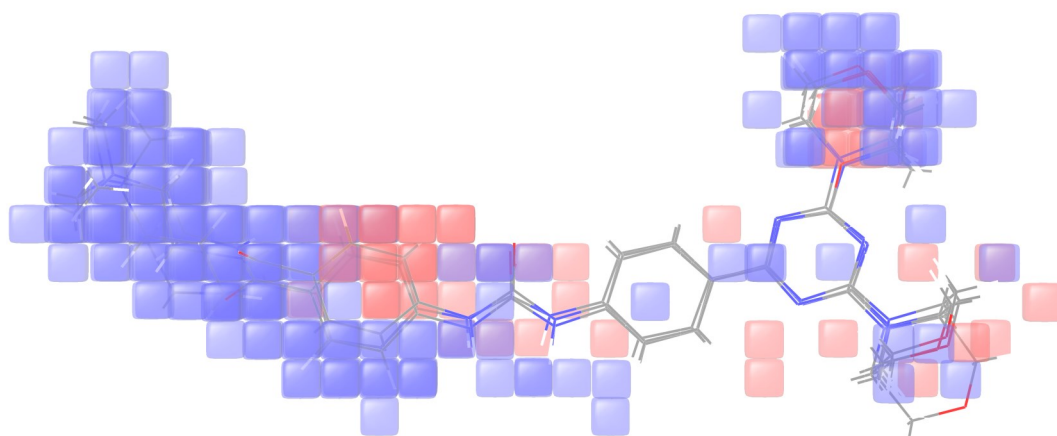
Table S5 MM-GBSA results

| | | pIC50 | dG |
|---------------|----------------|-------|---------|
| PI3K α | LAC (46228569) | 5.82 | -113.61 |
| | PKI-587 | 9.40 | -125.17 |
| | MAC (53379513) | 9.70 | -123.75 |
| | | pIC50 | dG |
| mTOR | LAC (56683919) | 7.84 | -122.30 |
| | PKI-587 | 9.38 | -151.39 |
| | MAC (44514208) | 9.52 | -136.76 |

Table S6 Molecular weight of the compounds (g/mol)

| CID | MW |
|----------|--------|
| 44473371 | 575.66 |
| 44513884 | 462.50 |
| 44513885 | 462.50 |
| 44513886 | 461.52 |
| 44513887 | 467.54 |
| 44513888 | 475.54 |
| 44514201 | 479.51 |
| 44514203 | 495.96 |
| 44514205 | 497.50 |
| 44514208 | 562.66 |
| 44514210 | 548.64 |
| 44514481 | 413.47 |
| 44515083 | 505.57 |
| 44515084 | 491.54 |
| 44515696 | 519.55 |
| 44516307 | 505.53 |
| 44516310 | 518.57 |
| 44516635 | 641.76 |
| 44516636 | 655.79 |
| 44516953 | 615.73 |
| 44517288 | 560.65 |
| 44548482 | 558.63 |
| 45379222 | 399.45 |
| 45379223 | 463.49 |
| 45379224 | 624.13 |
| 45379226 | 626.15 |
| 46228569 | 468.55 |
| 53379513 | 590.67 |
| 56660108 | 574.63 |
| 56660109 | 602.68 |
| 56663573 | 546.62 |
| 56667023 | 534.61 |
| 56670464 | 560.65 |
| 56673952 | 616.71 |
| 56677282 | 520.58 |
| 56677283 | 532.59 |
| 56683916 | 520.58 |
| 56683917 | 616.71 |

(A)



(B)

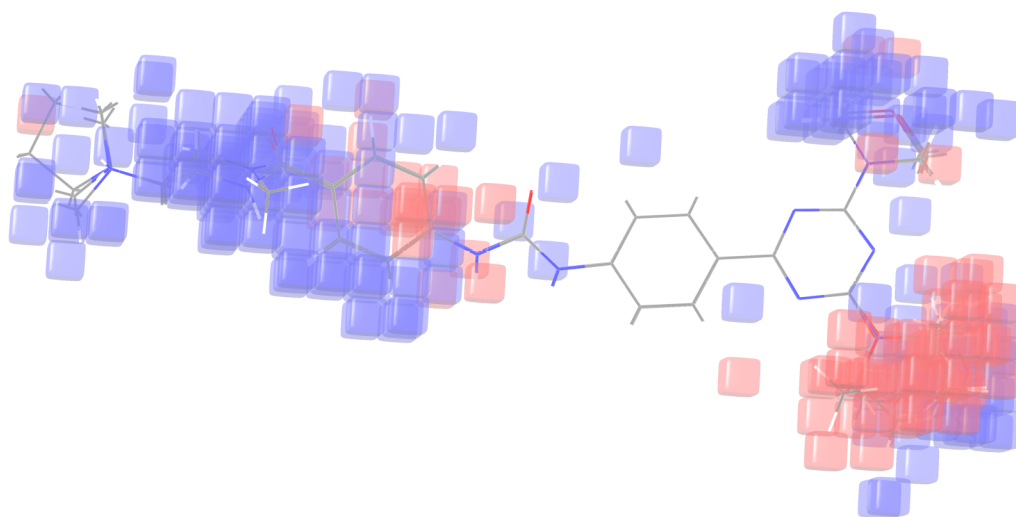


Figure S1 Combined effects and the 2D structures on aligned actives

(A) for PI3K α (B) for mTOR

Blue cubes: positive contribution to the inhibitive effects, red cubes: negative contributions.

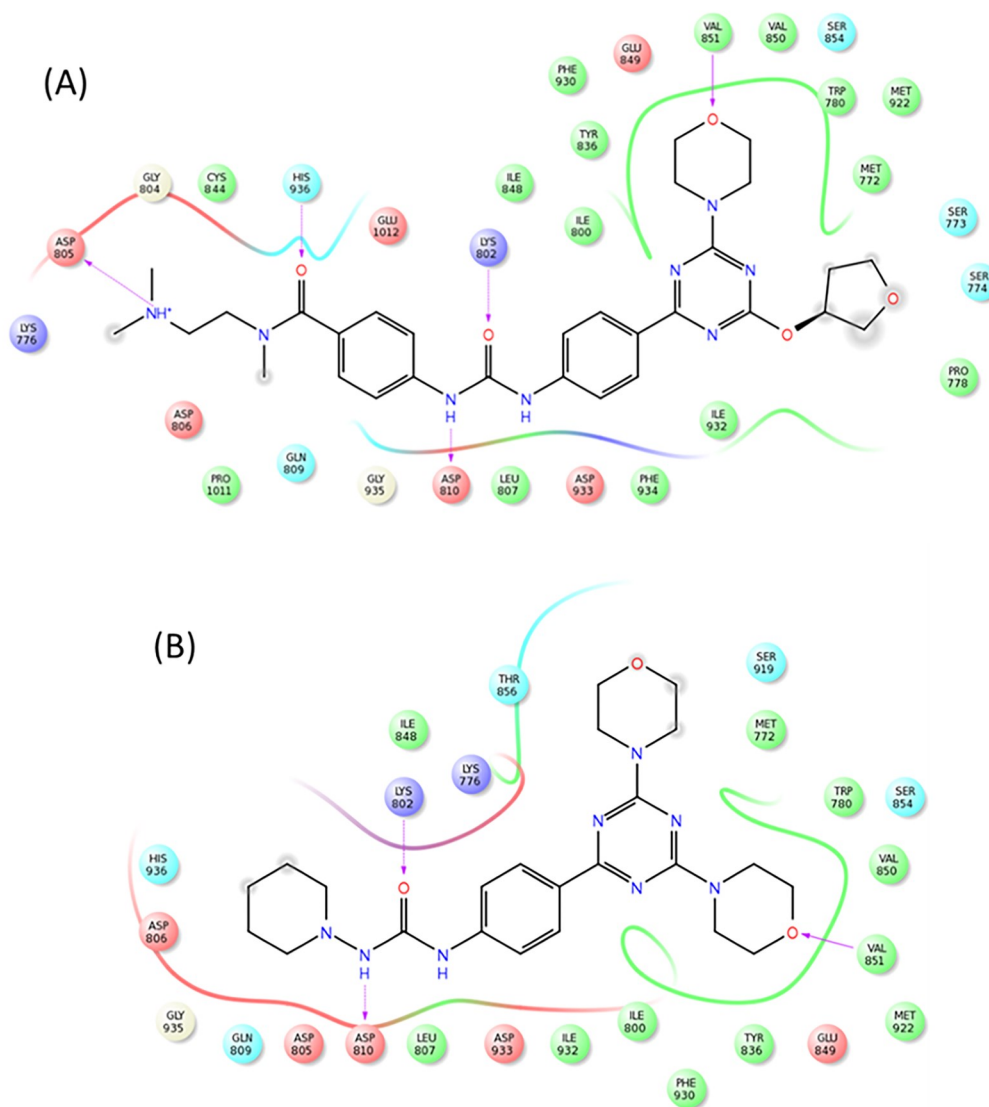


Figure S2 Ligand interaction diagram showing residues of PI3K α near ligands in docked complex structure for the MAC and LAC

The cut-off distance was 4 Å. (A) CID 53379513 (B) CID 46228569. Red circle: charged residue (negative), purple circle: charged (positive), blue: polar, green: hydrophobic, gray cloud: solvent exposure, purple arrow: hydrogen bond. Colored curved lines around ligands are protein “pocket” and the color represents the color of the closest residue.

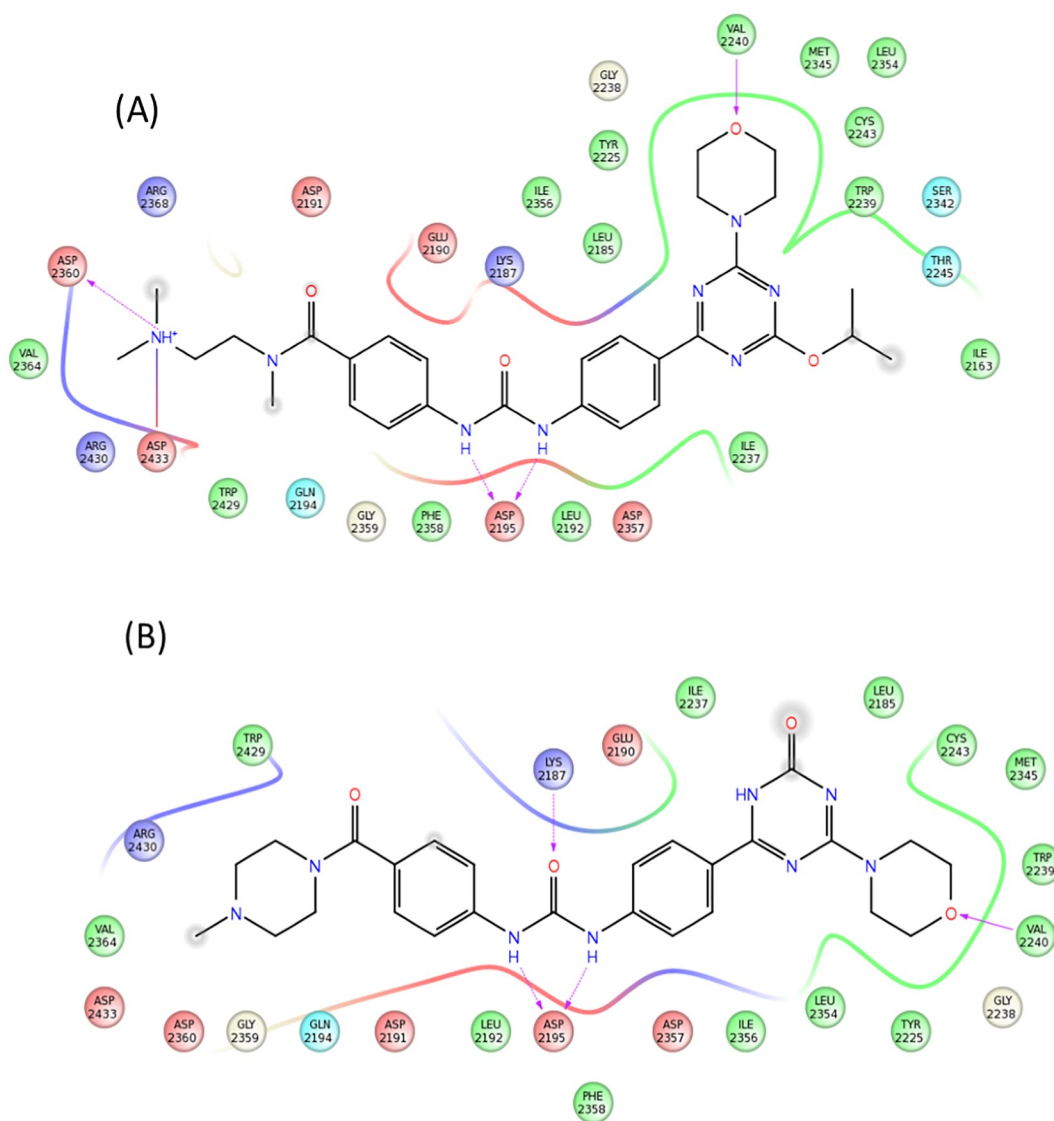
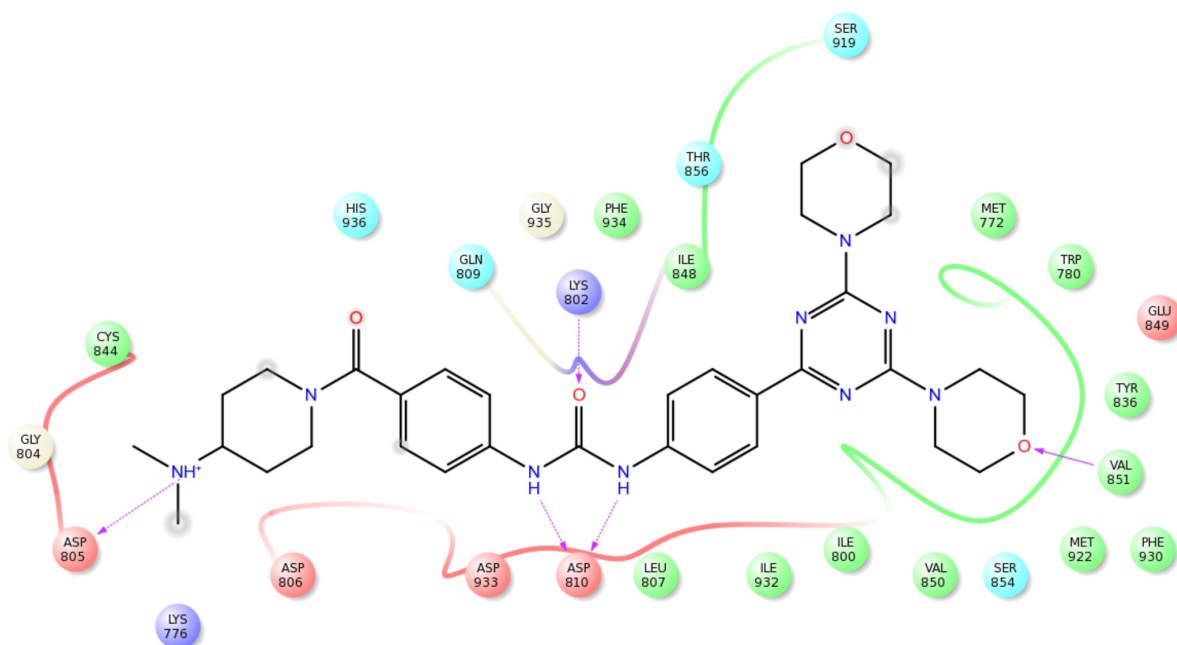


Figure S3 Ligand interaction diagram showing residues of mTOR near ligands in docked complex structure for the MAC and the LAC

(A) CID 44514208 (B) CID 56683919. The cut-off distance was 4 Å. Red circle: charged residue (negative), purple circle: charged (positive), blue: polar, green: hydrophobic, gray cloud: solvent exposure, purple arrow: hydrogen bond. Purple line: salt bridge. Colored curved lines around ligands are protein “pocket” and the color represents the color of the closest residue.

(A)



(B)

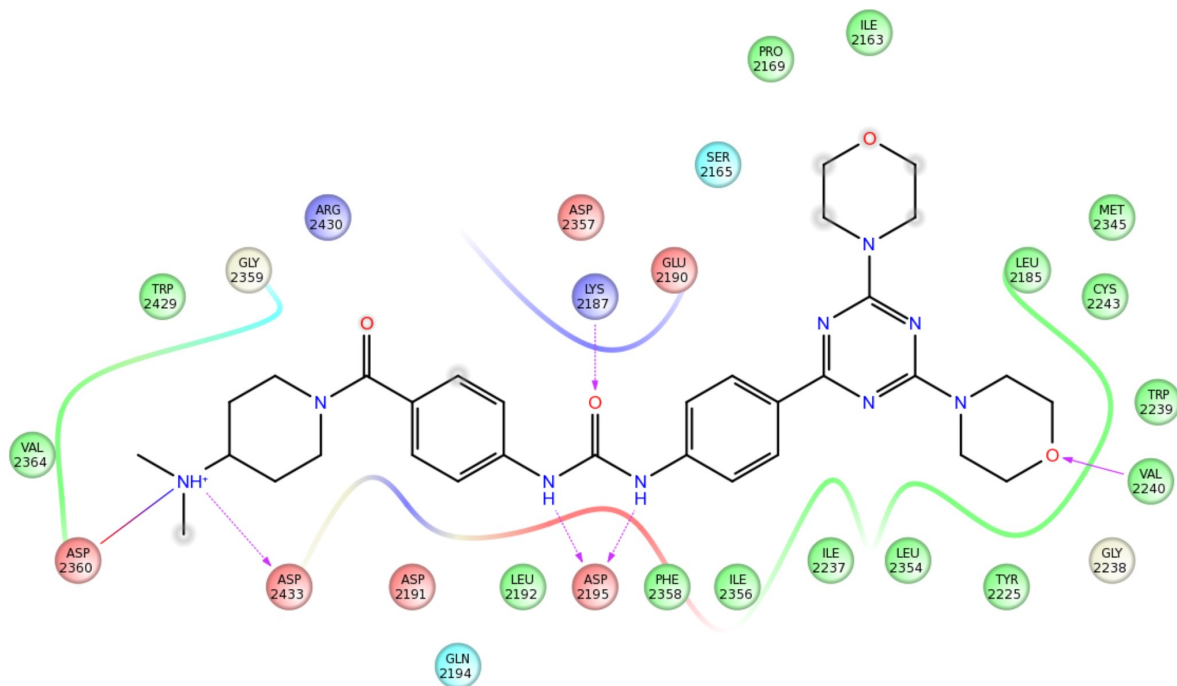
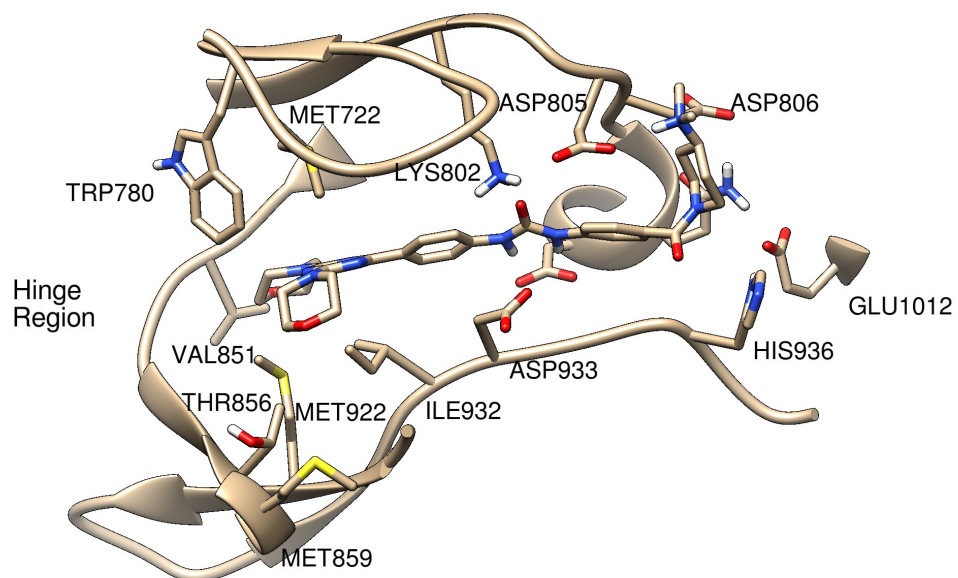


Figure S4 Ligand interaction diagram showing residues near PKI-587 in docked complex structure

(A) for PI3K α (B) for mTOR

The cut-off distance was 4 Å. Red circle: charged residue (negative), purple circle: charged (positive), blue: polar, green: hydrophobic, gray cloud: solvent exposure, purple arrow: hydrogen bond, purple line: salt bridge. Colored curved lines around ligands are protein “pocket” and the color represents the color of the closest residue.

A)



B)

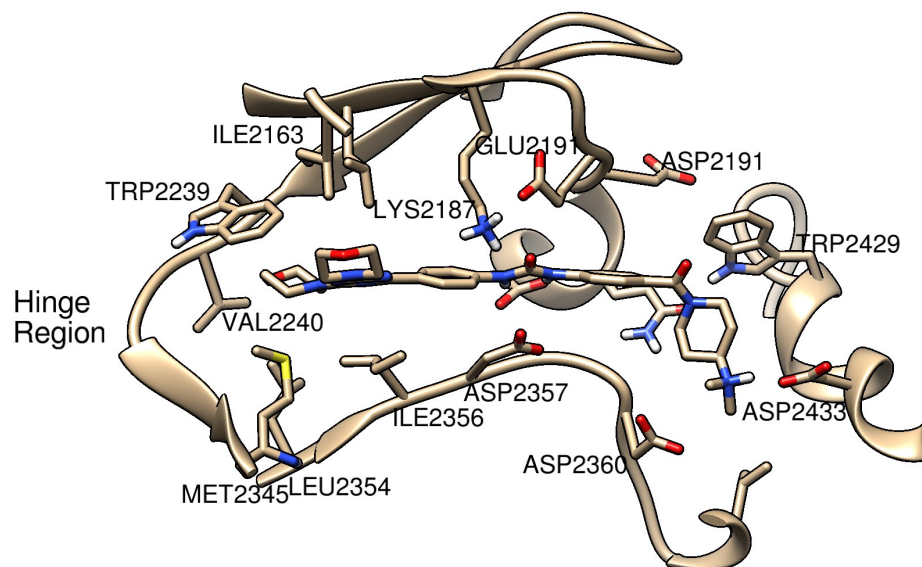


Figure S5 Docked structures of PKI-587

(A) for PI3Kα (B) for mTOR

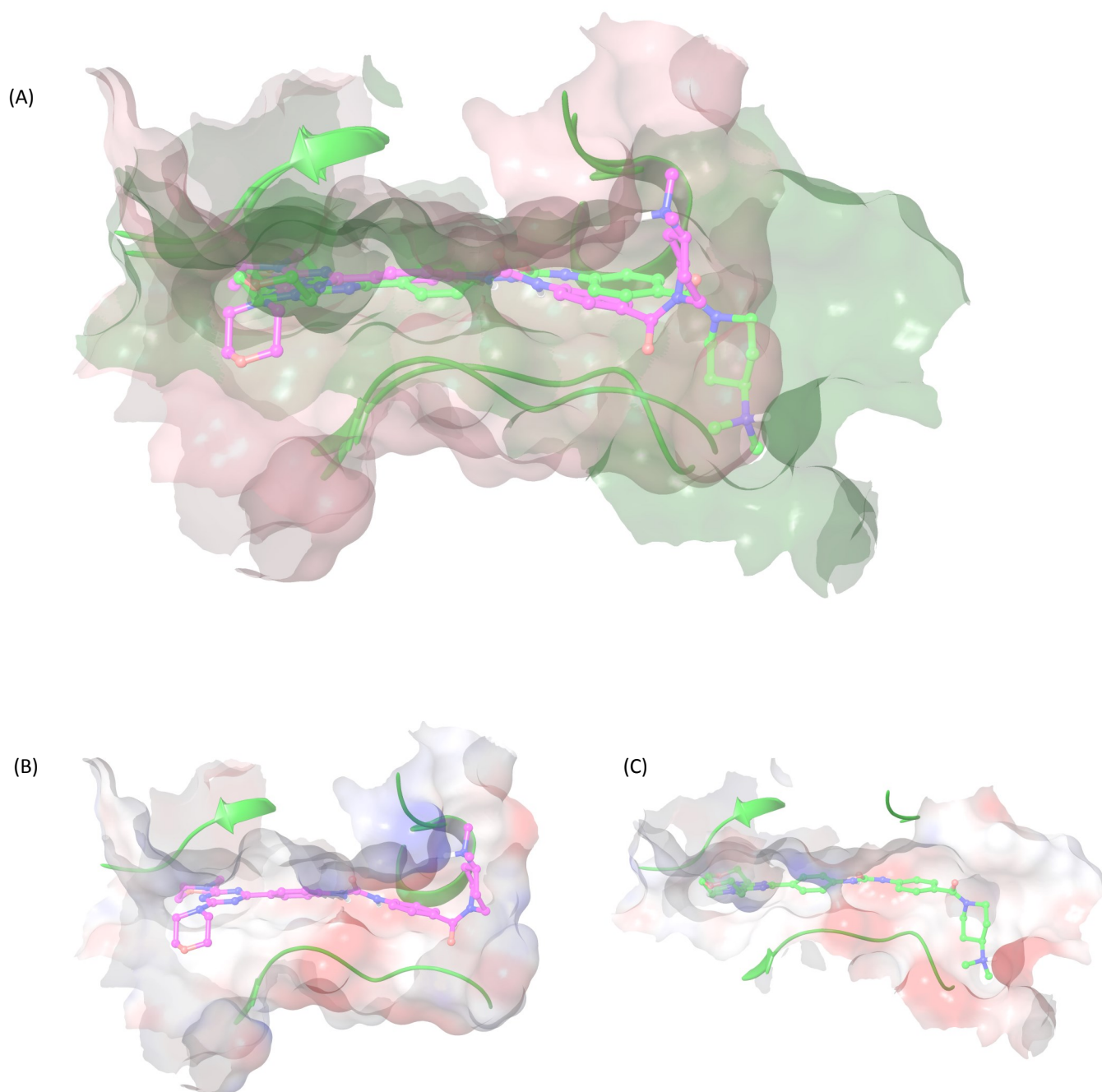


Figure S6 Binding site surface

(A) Image of superimposed binding site surface of the docked structures of PKI-587 for PI3K α and mTOR

Pink : PKI-587 with PI3K α green : : PKI-587 with mTOR (B) Electrostatic representation of binding site surface of the docked structures of PKI-587 for PI3K α blue:positively charged surface, red: negatively charged, white: non-polar. (C) Electrostatic representation of binding site surface of the docked structures of PKI-587 for mTOR Blue:positively charged ,red: negatively.

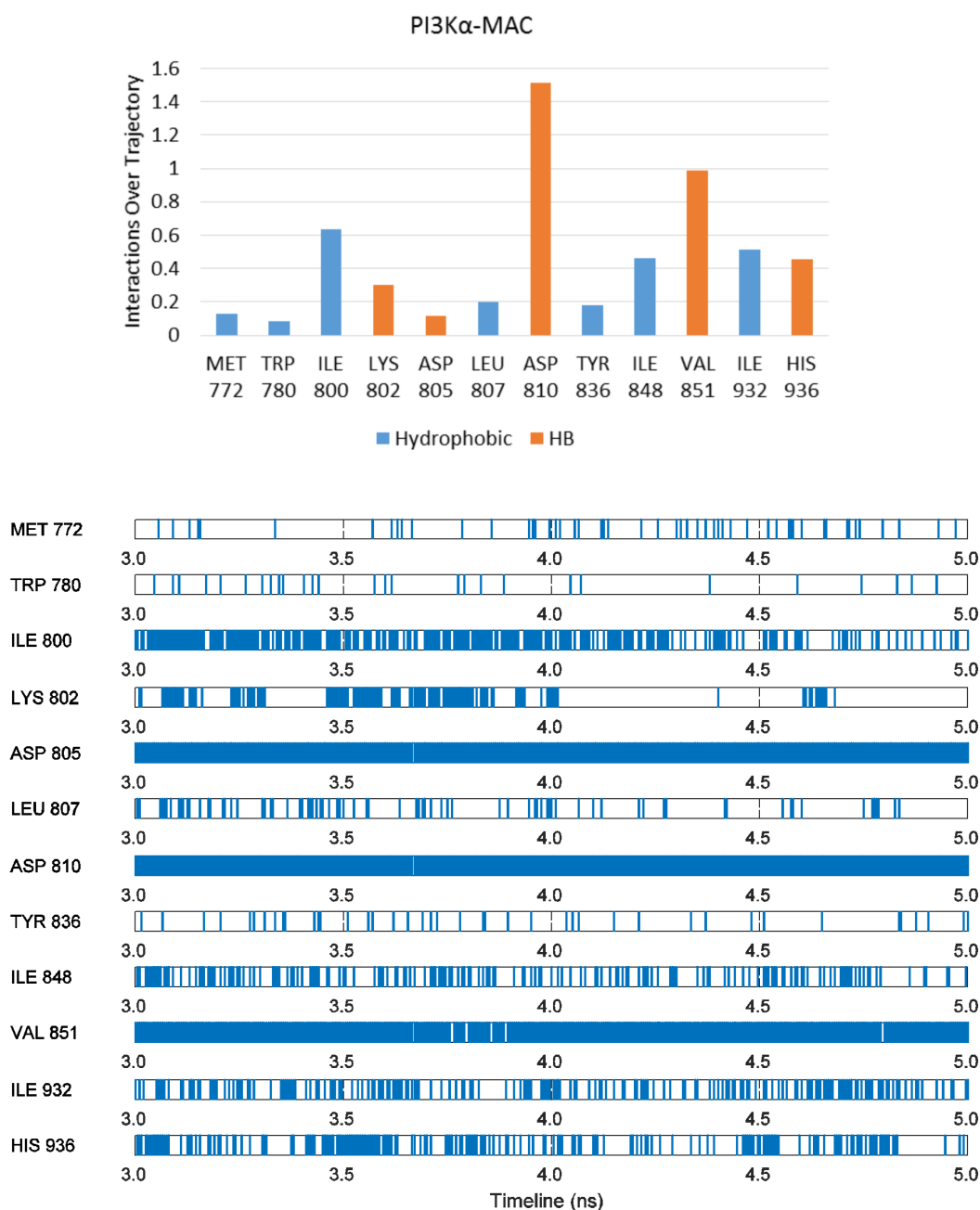


Figure S7 PI3K α -MAC Interaction over the course of trajectory and timeline representation in MD (3ns-5ns)

(A) PI3K α -MAC Interaction over the course of trajectory (Hydrophobic and HB) in MD during 3ns to 5ns. A value 0.6 indicates that for 60% of the simulation time the interaction existed. Values more than 1.0 indicates that the residue form multiple contacts (B) PI3K α -MAC timeline in MD during 3ns to 5ns.

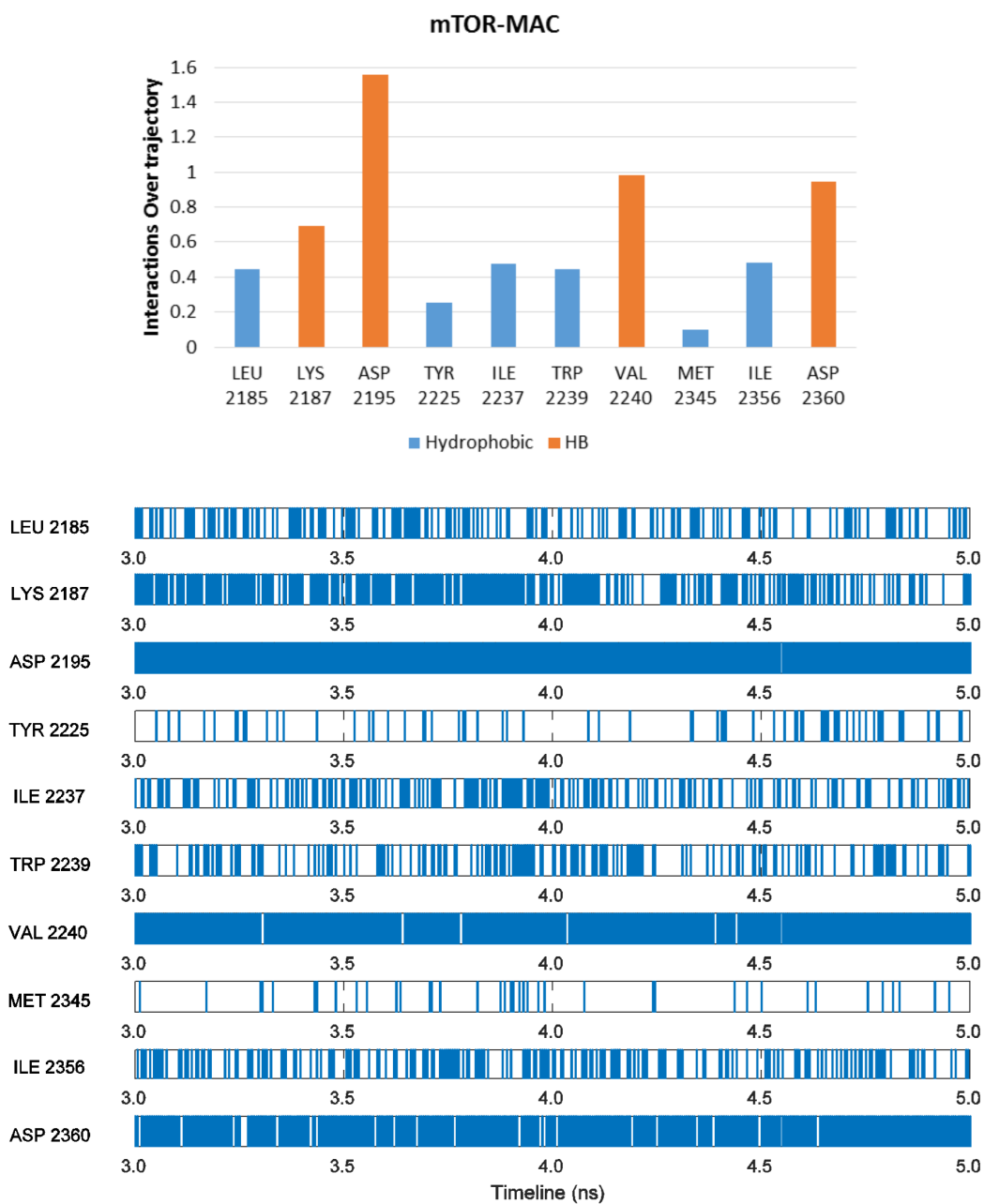


Figure S8 mTOR-MAC Interaction over the course of trajectory and timeline representation in MD (3ns-5ns)

(A) mTOR-MAC Interaction over the course of trajectory (Hydrophobic and HB) in MD during 3ns to 5ns. A value 0.6 indicates that for 60% of the simulation time the interaction existed. Values more than 1.0 indicates that the residue form multiple contacts. (B) mTOR-MAC timeline in MD during 3ns to 5ns.

Molecular Dynamics Simulation

Molecular dynamics (MD) simulations were applied to docked structures (docked poses) using Desmond (Schrödinger, LLC, New York, NY, 2013) to check the stability of the binding modes. The system was built by the system builder module in Maestro with the TIP3P water model and OPLS2005 force field. The initial coordinates for the MD computations were taken from the docked results. Na⁺ counter ions were added to neutralize the system and then 0.15M of NaCl was added. The total numbers of atoms in the system were 127426, 127464, 127459 for structure complexes, CID56683919 and mTOR, CID44514208 and mTOR, and PKI-587 and mTOR, respectively; and the total number of atoms were 119384, 118361, and 120152 for PKI-587 and PI3K α , CID 46228569 and PI3K α , and CID 53379513 and PI3K α , respectively. The shape of the simulation box was orthorhombic. The box size was calculated using a buffer distance of 10 Å between the solute structures and the simulation box boundary, and the volume of the box was minimized subsequently. The systems were minimized and pre-equilibrated by the Desmond default relaxation protocol which consists of a series of minimizations and short molecular dynamics simulations. The system was equilibrated and then moved to the production stage following the relaxation process. The Nose-Hoover chain thermostat method was used with relaxation time of 1ps to maintain the temperature at 300K and the Martyna-Tobias-Kline barostat method was used with relaxation time of 2 ps. The short-range coulombic interactions were truncated with cut-off value of 9 Å. The long-range coulombic interactions were controlled by the Smooth particle mesh Ewald method with tolerance of $1e^{-9}$. Total simulation times were 5 ns for all simulations. The trajectory recording interval was 10 ps.

Sequence alignment (BLASTP 2.3.0+ RESULTS [1])

BLASTP 2.3.0+with default parameters, Uniprot amino acid sequence of the catalytic domain of PI3Ka (sp|P42336|797-1068) and mTOR (sp|P42345|2182-2516) were aligned.

Query: sp|P42345|2182-2516 (mTOR)

Subject: sp|P42336|797-1068 ((PI3Kα)

Alignment statistics for match #1

| Score | Expect | Method | Identities | Positives | Gaps |
|----------------|--|------------------------------|--------------|--------------|-------------|
| 58.2 bits(139) | 3e-14 | Compositional matrix adjust. | 53/201 (26%) | 89/201 (44%) | 46/201(22%) |
| Query 4 | LLKGHEDLRQDERVMQLFGLVNTLLANDPTSLRKNLSIQRYAVIPLSTNSGLIGWVPHCD | 63 | | | |
| | + K +DLRQD +Q+ ++ + N LR + Y + + GLI V + | | | | |
| Sbjct 4 | IFKNGDDLRLQDMLTLQIIRIMENIWQNQGLDLR---MLPYGCLSIGDCVGLIEVVRNSH | 59 | | | |
| Query 64 | TLHALIRDYREKKKILLNIEHRIMLRMAPDYDHLTLMQKVEVFEHAVNNTAGDDLAKLLW | 123 | | | |
| | T+ + I+ + L+ A ++ TL Q W | | | | |
| Sbjct 60 | TI-----MQIQCKGGLKGLALQFNSHTLHQ-----W | 84 | | | |
| Query 124 | LKSPSSEVWFDRRTN-YTRSLAVMSMVGYYLGLGDRHPSNLMLDRLSGKILHIDFGDCFE | 182 | | | |
| | LK + +D + +TRS A + +ILG+GDRH SN+M+ + G++ HIDFG + | | | | |
| Sbjct 85 | LKDKNKGEIYDAAIDLFRSCAGYCVATFILGIGDRHNSNIMV-KDDGQLFHIDFGHFLD | 143 | | | |
| Query 183 | VAMTREKFP---EKIPFRLTR | 200 | | | |
| | ++KF E++PF LT+ | | | | |
| Sbjct 144 | --HKKKKFGYKRERVPFVLTQ | 162 | | | |

Range 2: 261 to 271GraphicsNext MatchPrevious MatchFirst Match

Alignment statistics for match #2

| Score | Expect | Method | Identities | Positives | Gaps |
|---------------|-------------|------------------------------|------------|------------|-----------|
| 15.4 bits(28) | 1.9 | Compositional matrix adjust. | 5/11 (45%) | 6/11 (54%) | 0/11 (0%) |
| Query 58 | WVPHCDTLHAL | 68 | | | |
| | W+ H HAL | | | | |
| Sbjct 261 | WIFHTIKQHAL | 271 | | | |

1)Altschul SF, Madden TL, Schäffer AA, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389-3402. doi: 10.1093/nar/25.17.3389

Information of corresponding residues in ATP-bound PI3K γ for mTOR

Val 2240 is the corresponding residue of Val 882 in PI3K γ . Asp 2195 is observed as forming HB with PI-103, The other dual inhibitor, in the crystal structure of mTOR–PI-103 complex [2]. The other residues corresponding to HB forming residues in ATP-bound PI3K γ are Ser 2165, Gln 2343, Asp 2357, and Lys 2187. Among them Asp2357 and Lys 2187 were within 4 Å from the ligand.

2) Yang H, Rudge DG, Koos JD, et al. (2013) mTOR kinase structure, mechanism and regulation. *Nature* 497:217–223. doi: 10.1038/nature12122